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Investigation for the role of CTX-III and microRNA-98 in diagnosis and treatment of osteoarthritis

W.-D. ZHENG[1], F.-L. ZHOU[2], N. LIN[2], J. LIU[1]

[1]Department of Orthopedic and Trauma Surgery, Jining No. 1 People's Hospital, Jining, Shandong, China
[2]Foundation College of Jining Medical University, Jining, Shandong, China

Abstract. – OBJECTIVE: Osteoarthritis is a joint degeneration and proliferative inflammatory disease caused by obesity, joint deformities, trauma, and other factors. C-terminal collagen (CTX) is associated with cartilage degradation, and healthy cartilage state is one of the factors that affect osteoarthritis. microRNA-98 (miRNA-98) plays a role in inflammation. This study aims to investigate the levels of CTX-III and miRNA-98 in patients with osteoarthritis and their potential clinical usage.

PATIENTS AND METHODS: Osteoarthritis was diagnosed according to the inclusion and exclusion criteria for osteoarthritis. Patients with osteoarthritis admitted to Jining No. 1 People's Hospital and healthy volunteers were included in this study. ELISA and Western blot analysis were used to detect levels of type III collagen CTX (CTX-III). Real time PCR was used to measure levels of miRNA-98 in the serum of both patients and healthy volunteers.

RESULTS: Levels of CTX-III protein in osteoarthritis patients were significantly higher than that of healthy volunteers (p = 0.0013). Levels of miRNA-98 in the serum of osteoarthritis patients were significantly higher compared to that of healthy volunteers (p = 0.0065). After treatment, levels of CTX-III protein and serum miRNA-98 in patients with osteoarthritis were significantly decreased (p = 0.014, p = 0.021). Levels of CTX-III protein and serum miRNA-98 in patients with osteoarthritis were significantly higher compared to that of healthy volunteers (p = 0.0013).

CONCLUSIONS: Both of the CTX-III and microRNA-98 are potential diagnostic indicators for the osteoarthritis.

Key Words:
Osteoarthritis, miRNA-98, CTX-III, Biomarker.

Introduction

Osteoarthritis is chronic articular cartilage degeneration caused by joint deformities, congenital joint abnormalities, aging, trauma, and obesity[1,2]. It is also known as hypertrophic cartilage arthritis, bone and joint disease, or degenerative cartilage disease[3,4]. It causes not only pain but also burden[5]. Therefore, the study of osteoarthritis is of great significance for both theoretical and clinical study.

Early diagnosis of osteoarthritis is very important[6,7]. Classical X-ray detection cannot detect any bone or joint injury[8,9]. Magnetic resonance imaging (MRI) and computed tomography (CT) examination are both time-consuming and high-cost[10,11]. Therefore, simpler but effective diagnosis of osteoarthritis is needed.

CTX-II is widely studied as a biomarker of osteoarthritis. Collagen is the most abundant protein in animals[12]. There are two major types of collagen, type II collagen, and type III collagen. As CTX-II has smaller molecular weight, it is widely used as a biomarker of osteoarthritis[13]. However, it is difficult to detect CTX-II by Western blot[3]. CTX-III has a relatively stable cleavage pattern[13]. The possibility of using CTX-III as a biomarker of osteoarthritis was investigated. microRNA is a type of small RNA. Although not encoding proteins, microRNA has a wide range of biological effects[14,15]. For example, serum microRNA levels are closely related with diseases particularly sepsis[16]. miRNA-98 is closely related to the body's immune status[5] and might be used as a biomarker of osteoarthritis.

Therefore, levels of CTX-III protein and serum miRNA-98 in patients with osteoarthritis and

Corresponding Author: Jian Liu, MD; e-mail: xumaioi@163.com

healthy volunteers were measured to provide a theoretical basis for the diagnosis and treatment of osteoarthritis.

Patients and Methods

Patients

Osteoarthritis patients of Jining No. 1 People's Hospital (Shangdong, China) from January 2013 to January 2016 were included, while healthy volunteers were used as controls. Osteoarthritis inclusion criteria were previous reported13. The clinical parameters, including disease history, no knee surgery history, having clinical symptoms of osteoarthritis, and the relevant imaging diagnosis, were evaluated in this study. The exclusion criteria included secondary osteoarthritis, history of joint surgery, and rheumatoid arthritis. This study was approved by the Research Ethics Committee of our Institution. The informed consents was signed from all the subjects.

A total of 20 patients with osteoarthritis (10 males and 10 females) were included with an average age of 60 ± 8 years. A total of 20 healthy volunteers (10 male and 10 female) were included as controls with an average age of 58 ± 9 years. All subjects have no family history of heart disease or brain disease. Osteoarthritis patients underwent six-month glucosamine sulfate treatment. Joint pain relief was used as a simple assessment of efficacy.

Blood Sample Collection

Total of 5 ml fasting blood was collected. The supernatant was collected after centrifugation and divided into two parts, one for ELISA and Western blot detection of CTX-III, and the other one for the detection of microRNA-98 (miRNA-98).

ELISA

Serum CTX-III levels were measured according to the conventional ELISA methods. Briefly, serum was kept in 96-well plates at 4°C overnight. CTX-III antibodies were then added for specific binding. After washing away the excess test sample, the secondary antibody was added. A microplate reader (Mode: MK3, Thermo Scientific, Waltham, MA, USA) was used to record optical density (OD) values.

Western Blot

Serum CTX-III levels were measured according to the conventional Western blot methods. Briefly, serum samples were mixed with 6x loading buffer and resolved by SDS-polyacrylamide gel electrophoresis. Proteins were then transferred to nitrocellulose filter membrane (NC, Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) membrane. The membrane was incubated with mouse anti-human CTX-III monoclonal antibodies[17] for 120 min. The membrane was washed and incubated with goat anti-mouse secondary antibodies for 60 min. After developing and fixing, the gel imaging system was used to record the results. Image J software (version 2.0, Image J, Scion Image, Frederick, MD, USA) was used to quantify the band density. Actin was used as the internal reference gene.

Real-Time PCR

Serum miRNA-98 levels were detected using Real Time PCR. The sequences of primers for miRNA-98 and the internal control, actin were: 5'-AGGCCUCGCUGUUCUCUAUGGC-3 'and 5'-UUAUUCCUAUGUGAUUCUACU-3'; 5'-GCUCACUCAUAUAGGGUGGAGC-3 'and 5'-AUCGUGGCGCACGGCGGGGACA-3'. Briefly, RNA was extracted and reverse transcripted into complementary DNA (cDNA) for Real Time PCR. The PCR products were subjected to agarose gel electrophoresis. Image J software was used to quantify gel images and Actin was used as internal control.

Statistical Analysis

SPSS 13.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Data are expressed as mean ± standard deviation. The Student's *t*-test was used to compare the differences between the two groups. Tukey's post-hoc test was used to validate the ANOVA for comparing measurement data between groups. $p < 0.05$ was considered as statistical significance.

Results

Enzyme-Linked Immunosorbent Assay (ELISA) Results of Serum CTX-III

As shown in Figure 1, ELISA results showed that levels of CTX-III in patients with osteoarthritis were significantly higher than that of the healthy volunteers ($p = 0.0013$).

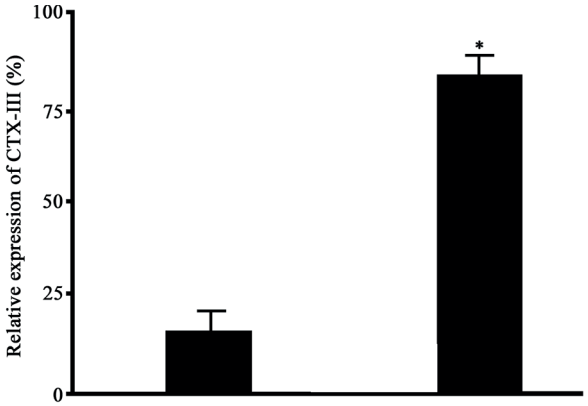

Figure 1. ELISA results of levels of serum CTX-III. *$p < 0.05$.

Western Blot Detection of CTX-III Protein

Western blot results (Figure 2) showed that levels of CTX-III in patients with osteoarthritis were significantly higher than that of the healthy volunteers ($p = 0.0028$).

Real Time PCR Measurement of MiRNA-98 Levels

As shown in Figure 3, Real-Time PCR results showed that levels of miRNA-98 in patients with osteoarthritis were significantly higher than that of the healthy volunteers ($p = 0.0065$).

Levels of CTX-III in Patients With Osteoarthritis Declined After Treatment

As shown in Figure 4, after treatment, levels of CTX-III in patients with osteoarthritis significantly declined ($p = 0.014$).

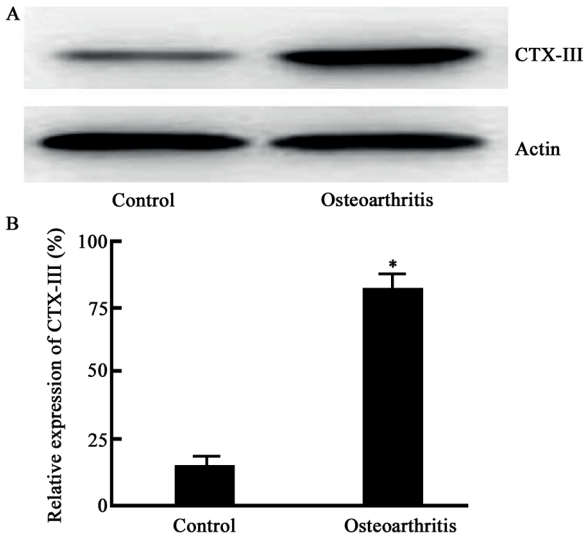

Figure 2. Western blot results of CTX-III protein. *$p < 0.05$.

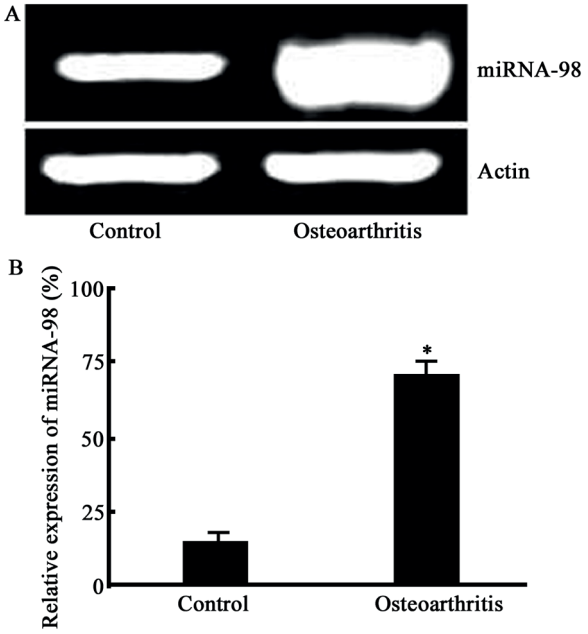

Figure 3. Real-time PCR measurement of miRNA-98 levels. *$p < 0.05$.

Levels of MiRNA-98 in Patients With Osteoarthritis Declined After Treatment

As shown in Figure 5, after treatment, serum miRNA-98 levels in patients with osteoarthritis significantly decreased ($p = 0.021$).

Discussion

Diagnosis of osteoarthritis is of great significance[1]. However, it is difficult to diagnose osteoarthritis mainly because of the lack of biomarkers of osteoarthritis[5]. Therefore, this work investigated the potential biomarkers of osteoarthritis.

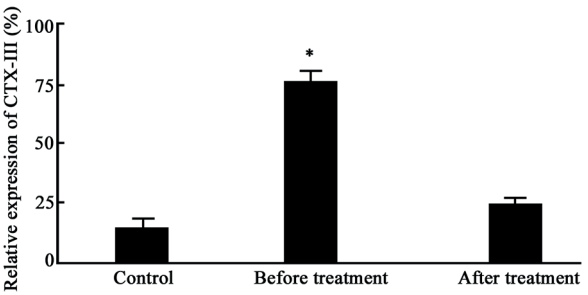

Figure 4. Levels of CTX-III before and after treatment. *$p < 0.05$.

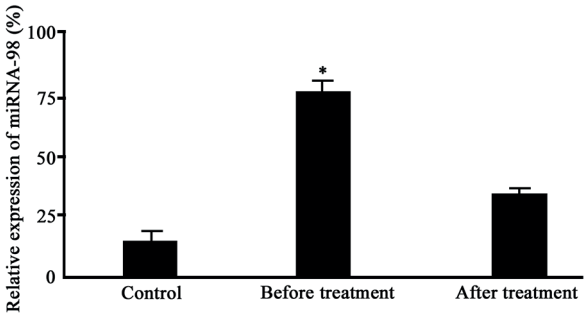

Figure 5. Levels of miRNA-98 in patients with osteoarthritis declined after treatment. *$p < 0.05$.

Existing research suggests that CTX-II is a biomarker for osteoarthritis[13]. However, since the CTX-II has been degraded inside the body, it is hard to show the actual levels of serum CTX-II, which limits its clinical application and reduces its diagnostic accuracy. Because CTX-III and CTX-II are high homologous, so we speculate that CTX-III can also be used as a biomarker for osteoarthritis. Our result also proved this suggestion, which is consistent with previous studies[16-18]. Of note, CTX-III is much more stable than CTX-II. Thus, CTX-III has a better potential as a biomarker of osteoarthritis. microRNA has a variety of biological effects[19,20]. It has been shown that miRNA-98 is related to inflammation[21]. This study investigated the correlation between osteoarthritis and CTX-III and miRNA-98. The results suggested that miRNA-98 levels were closely correlated with osteoarthritis. Moreover, the cost for detecting the CTX-III and miRNA-98 levels is relatively lower and the effectiveness is relatively higher, therefore, these two biomarkers are needed to be studied in the future study.

Although the present work received some interesting findings, there were a few limitations. Firstly, it is worth to mention that the number of subjects was little which may not reflect the status of the entire osteoarthritis patients. Secondly, levels of serum CTX-III and miRNA-98 might be affected by other medicines, such as non-steroidal anti-inflammatory drugs, glucocorticoids, taken by patients before the enrollment of this study, which may interfere with our results. In the future research, we would address this point in greater detail, and investigate whether these drugs affect the levels of CTX-III and miRNA-98.

The study population is small and would be enlarged in the following research.

Conclusions

We showed that the levels of serum CTX-III and miRNA-98 in patients with osteoarthritis were significantly higher than that of healthy volunteers, suggesting that CTX-III and microRNA-98 might be used as potential diagnostic indicators of osteoarthritis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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